PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P 64472	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/EP2004/000109	International filing date *(day/month/year)* 09.01.2004	Priority date *(day/month/year)* 11.07.2003
International Patent Classification (IPC) or national classification and IPC C12N5/06, C12N5/08		
Applicant BLASTICON BIOTECHNOLOGISCHE FORSCHUNG GMBH et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 8 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 6 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☒	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☒	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
01.10.2004	28.04.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Noë, V Telephone No. +31 70 340-4181

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/000109

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-65 as originally filed

Claims, Numbers

1-37 received on 18.03.2005 with letter of 17.03.2005

Drawings, Sheets

1/12-12/12 as originally filed

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☒ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☒ the claims, Nos. 38-39
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/000109

Box No. II Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
   - ☐ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).
   - ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).

2. ☒ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 32-37

   because:

   ☒ the said international application, or the said claims Nos. 32-37 (for industrial applicability) relate to the following subject matter which does not require an international preliminary examination (specify):

   see separate sheet

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos.

   ☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

   ☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

   ☐ See separate sheet for further details

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-37
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-37
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-31
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

III. Non-establishment of opinion (Continuation)

1 Claims 32-37 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv)PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

V. Reasoned statement (Continuation)

2 CITATIONS

Reference is made to the following documents:

D1: WO 2004/007701 A (KREMER BERND KARL FRIEDRICH ; FAENDRICH FRED (DE); RUHNKE MAREN (DE);) 22 January 2004 (2004-01-22)

D5: LOPEZ M ET AL: "Infusion of large quantities of autologous blood monocyte-derived macrophages in two cancer patients did not induce increased concentration of IL-6, TNF-alpha, soluble CD14 and nitrate in blood plasma" EUROPEAN CYTOKINE NETWORK, vol. 5, no. 4, 1994, pages 411-414,

3 NOVELTY (Art. 33(2) PCT)

3.1 Claim 13 for a product, namely monocytic cells expressing CD3 and CD14 defined in terms of a process of manufacture is admissible only if the product as such fulfills the requirements of patentability, i.e. inter alia that it is novel and inventive. A product is not rendered novel merely by the fact that it is produced by means of a new process. Since none of the cited prior art documents disclose CD3 and CD14 expressing monocytic cells and these two cell markers are known to be specific for different populations of blood cells namely for T-lymphocytes and monocytes, this cell population is considered to be novel.

3.2 The present application satisfies the criterion set forth in Article 33(2) PCT because

the subject-matter of claim 1-37 is new in the sense in respect of the prior art as defined in the regulations (Rule 64(1)-(3) PCT).

4 INVENTIVE STEP (Art. 33(3) PCT)

4.1 For inventive step analysis of claim 1, D5 is considered to represent the closest prior art and discloses a process for preparation of cells for the treatment of cancer by culturing monocytic cell in a culture medium comprising GM-CSF followed by addition of γ-IFN. The difference with the application is that a process for preparation of cells for the treatment of diseases associated with disturbed self-tolerance is claimed comprising culturing monocytic cells in the presence of M-CSF and or followed γ-IFN.

4.2 The problem to be solved by the present application might therefore be regarded as the provision of an alternative process for the preparation of monocytic cells for the treatment of an alternative disease.

4.3 The solution provided by the present application is a process for preparation of cells for the treatment of diseases associated with disturbed self-tolerance comprising culturing monocytic cells in the presence of M-CSF and or followed γ-IFN. This solution is considered to involve an inventive step because none of the cited prior documents discloses nor suggests the cultivation of blood-derived monocytes with both M-CSF and γ-IFN, in order to provide monocytic cells for the prevention and/or treatment of diseases associated with disturbed self-tolerance and this would not be obvious for the person skilled in the art.

4.4 The cells of claims 13-15 are also considered to involve an inventive step because none of the cited prior art documents suggests the existence of CD3 AND CD14 expressing monocytic cells. These two cell markers are known to be specific for different populations of blood cells namely for T-lymphocytes and monocytes. Therefore, preparation and identification of cells expressing both markers would not be obvious for the skilled person. Moreover, these cells have an unexpected effect : they are shown to be suitable for the prevention and/or treatment of diseases associated with disturbed self-tolerance.

4.5 For the same reasons indicated above (see 5.4) also a cell preparation (claim 15) or

pharmaceutical composition comprising the CD3 and CD 14 expressing cells of claims 13 or 14 (claim 16-19), use of these cells in the preparation of a medicament for treating diseases associated with disturbed self-tolerance (claims 20-25), use of the cells for in vitro generating and/or propagating regulatory T-cells (claim 26-27), the process of generating regulatory T-cells using the cells of claims 13-14 (claims 28-30), the method for detection and selection of the cells of claim 31 and a method of treatment of diseases associated with disturbed self-tolerance by administering the cells (claims 32-37) are considered to be inventive.

4.6 The present application therefore satisfies the criterion set forth in Article 33(3) PCT and the subject-matter of claims 1-37 does involve an inventive step (Rule 65(1)(2) PCT).

5 INDUSTRIAL APPLICABILITY (Art. 33(4) CT)

5.1 For the assessment of the present claims 32-37 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

VI. Certain documents cited (Continuation)

6.1 Certain published documents

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO2004/007701	22.01.2004	11.07.2003	12.07.2002

VIII. Certain Observations (Continuation)

7.1 Claim 28 comprises an optional feature which is considered to have no limiting effect on the scope of the claim and is regarded to be entirely optional. Therefore, to fulfill the requirements of Art. 6 PCT optional features should be avoided. If the applicant wishes to claim the optional feature, a dependent claim should be drafted.

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EPO - DG 1 17. 13. 2005
18. 03. 2005
(82)

Claims

1. A process for the preparation of cells for the prevention and/or treatment of diseases associated with disturbed self-tolerance in a patient, characterised in that

   a) monocytes isolated from the blood of the patient to whom the cells are to be administered are in vitro multiplied in a suitable culture medium which contains the cellular growth factor M-CSF;

   b) the monocytes are cultivated simultaneously with or following step a) in a culture medium containing γ-IFN; and

   c) the cells formed in step b) are obtained by separating the cells from the culture medium.

2. A process according to claim 1 characterised in that the monocytes are of human origin.

3. A process according to claims 1 or 2 characterised in that the monocytes are isolated from the blood in such a manner that next to the monocytes also lymphocytes are present in an amount of at least 10% by reference to the total cell number in the isolate.

4. A process according to claims 1 to 3, characterised in that the cells formed in step b) or obtained in step c) are selected by binding to the antibody produced by the hybridoma cell line DSM ACC2542.

5. A process according to claims 1 to 4, characterised in that among the cells formed in step b) or obtained in step c) of claim 1 or obtained in the selection step according to claim 4, those cells are selected which co-express the antigens CD3 and CD14 on their cell surface.

6. A process according to claims 1 to 5, characterised in that the M-CSF concentration in the culture medium is 1 to 20 µg/l.

7. A process according to claims 1 to 6, characterised in that, subsequent to step a) the monocytes are cultivated for 24 to 72 hours in a culture medium containing γ-IFN, the cultivation in the presence of γ-IFN beginning 3 to 6 days after the beginning of cultivation step a).

8. A process according to claim 7, characterised in that the γ-IFN concentration in the culture medium is 0.1 to 20 ng/ml.

9. A process according to claims 1 to 8 characterised in that the total cultivation period in steps a) and b) is 4 to 8 days.

10. A process according to claims 1 to 9 characterised in that subsequent to step c) of claim 1, or subsequent to the selection steps according to claims 4 and 5, the cells are suspended in a suitable cell culture medium or in a PBS or NaCl solution.

11. A process according to claims 1 to 10 characterised in that the cells are suspended in a freezing medium and are subsequently deep frozen.

12. A process according to claim 11 characterised in that the freezing medium comprises fetal calf serum (FCS) or human AB0 compatible serum and DMSO.

13. Cells co-expressing the antigens CD3 and CD14 on their cell surface for the prevention and/or treatment of diseases associated with disturbed self-tolerance in patient, obtainable by any of the processes according to claims 1 to 12.

14. Cells according to claims 13 characterised in that they are of human origin.

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15. Cell preparation containing the cells according to claims 13 or 14 in a suitable medium.

16. Pharmaceutical composition containing cells of monocytic origin, co-expressing the antigens CD3 and CD14 on their cell surface, obtainable by the process of claims 1 to 12 for the prevention and/or the treatment of diseases associated with disturbed self-tolerance in a patient.

17. Pharmaceutical composition containing the cells according to claims 13 or 14 or the cell preparation according to claim 15.

18. Pharmaceutical composition according to claims 16 and 17 for the prevention and/or the treatment of autoimmune diseases.

19. Pharmaceutical composition according to claims 16 and 17for the prevention and/or the treatment of allergies.

20. Use of the cells according to claims 13 to 14 or the cell preparation according to claim 15 for manufacturing a pharmaceutical composition for the prevention and/or treatment of diseases associated with disturbed self-tolerance.

21. Use according to claim 20 for the prevention and/or treatment of autoimmune diseases.

22. Use of claim 21, characterised in that the autoimmune disease is one or more of the diseases selected from rheumatic diseases with autoimmune features, diabetes mellitus, autoimmune diseases of the blood and blood vessels, autoimmune diseases of the liver, autoimmune diseases of the thyroid, autoimmune diseases of the central nervous system, and bullous skin diseases.

23. Use according to claim 20 for the prevention and/or treatment of allergies.

24. Use according to claim 23, characterised in that the allergy is selected from allergies induced by non-self proteins, organic substances and/or inorganic substances.

25. Use according to claim 24, characterised in that the allergy is selected from hayfever and/or allergies induced by drugs, chemicals, viruses, bacteria, fungi, food components, metals, gases, cat skin scale and/or animal hair.

26. The use of self-tolerance inducing cells according to claims 13 to 14 or the cell preparation of claim 15 for *in vitro* generating and/or propagating autologous regulatory T-lymphocytes.

27. The use according to claim 26, wherein the regulatory T-lymphocytes co-express the antigens CD4 and CD25 on their cell surface.

28. A process for the generation and/or propagation of autologous regulatory T-lymphocytes, characterised in that

a) self-tolerance inducing cells according to claims 13 to 14 or a cell preparation according to claim 15 are co-cultivated with an autologous T-lymphocyte preparation, and

b) the regulatory T-lymphocytes are optionally obtained from the culture medium.

29. A process according to claim 28, characterised in that the regulatory T-lymphocytes co-express the antigens CD4 and CD25 on their cell surface.

30. A process according to claims 28 or 29, characterised in that the regulatory T-lymphocytes are obtained from the culture medium by FACS sorting.

31. The use of the antibodies produced by the hybridoma cell line DSM ACC2542 for the detection and/or selection of cells obtained by the process of claims 1 to 12 suitable for the prevention and/or treatment of diseases associated with disturbed self-tolerance in a patient.

32. A method for the prevention and/or treatment of diseases associated with disturbed self-tolerance in a patient, characterised in that a pharmaceutically effective amount of the autologous cells according to claims 13 to 14 or the

autologous cell preparation according to claim 15 is administered to the patient.

33. The method according to claim 32, for the prevention and/or treatment of autoimmune diseases.

34. The method according to claim 33, wherein the autoimmune disease is one or more of the diseases selected from rheumatic diseases with autoimmune features, diabetes mellitus, autoimmune diseases of the blood and blood vessels, autoimmune diseases of the liver, autoimmune diseases of the thyroid, autoimmune diseases of the central nervous system, and bullous skin diseases.

35. The method of claim 32 for the prevention and/or treatment of allergies.

36. The method of claim 35, wherein the allergy is selected from allergies induced by non-self proteins, organic substances and/or inorganic substances.

37. The method of claim 36, wherein the allergy is selected from hayfever and/or allergies induced by drugs, chemicals, viruses, bacteria, fungi, food components, metals, gases, animal skin scale, hair and/or animal excreta.